

## Claims

1. On-line detection method comprising the on-line coupling of the effluent of a fractionation step to a mass spectrometer, which method comprises the addition of a controlled amount of an affinity molecule to said effluent, whereby the affinity molecules bind analytes in the effluent, followed by a separation step using a restricted-access support, whereby the analyte-affinity molecule complex is permeated, followed by a suitable dissociation step to dissociate the analyte-affinity molecule complex, followed by a second separation step in which the dissociated analyte and affinity molecules are separated, followed by detection of the analyte using the mass spectrometer.
2. On-line detection method according to claim 1, in which the second separation step is carried out using a restricted-access support, in which the affinity molecule is retained, followed by elution of the analyte from the restricted-access support using a suitable carrier stream, and directing the eluted stream to the mass spectrometer.
3. On-line detection method according to claim 1, in which the second separation step is carried out using a hollow fiber support, whereby the analyte is permeated and the permeate is directed to the mass spectrometer.
4. On-line detection method according to any of the preceding claims, in which the dissociation step is a low pH shock, contacting with a high ionic strength solution, contacting with an organic solvent and/or contacting with a chaotropic reagent.
5. Method according to any of the preceding claims in which the fractionation step is a liquid chromatography separation, a capillary electrophoresis step or a combinatorial chemistry system, which is optionally followed by a separation step which removes the high molecular weight background.

6. Method according to claim 5, in which the liquid chromatography separation step is a HPLC, a reversed phase HPLC, a CE, a CEC, a IEF or a MEKC step.

7. Method according to any one of the preceding claims, in which the  
5 mass spectrometer is of the type chosen from the group consisting of electrospray ionization type, atmospheric pressure ionization type, quadrupole type, magnetic sector type, time-off-flight type, MS/MS, MS<sup>n</sup>, FTMS type, ion trap type and combinations thereof.

8. Method according to any one of the preceding claims, in which the  
10 mass spectrometer is set to detect ions of a selected single m/z trace, selected multiple m/z traces, in scanning mode or any sequential mode.

9. The method of any of the preceding claims wherein the affinity molecule is an affinity protein.

10. The method of any of the preceding claims wherein the affinity  
15 molecule is an orphan receptor.

11. Compound detected by the method of any one of the preceding claims.

12. The use of a compound of the preceding claim as a ligand for affinity molecules.

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